Title

Identification of the major proteins that promote neuronal process outgrowth on Schwann cells in vitro.

Journal

The Journal of cell biology, 107(1)

ISSN

0021-9525

Authors

Bixby, JL
Lilien, J
Reichardt, LF

Publication Date

1988-07-01

DOI

10.1083/jcb.107.1.353

Peer reviewed

Identification of the Major Proteins That Promote Neuronal Process Outgrowth on Schwann Cells In Vitro

John L. Bixby,* Jack Lilien,‡ and Louis F. Reichardt*

*Department of Physiology and the Howard Hughes Medical Institute, University of California, San Francisco, California 94143-0724; and ‡Department of Zoology, University of Wisconsin, Madison, Wisconsin 53706

Abstract. Schwann cells have a unique role in regulating the growth of axons during regeneration and presumably during development. Here we show that Schwann cells are the best substrate yet identified for promoting process growth in vitro by peripheral motor neurons. To determine the molecular interactions responsible for Schwann cell regulation of axon growth, we have examined the effects of specific antibodies on process growth in vitro, and have identified three glycoproteins that play major roles. These are the Ca^{2+}-independent cell adhesion molecule (CAM), L1/Ng-CAM; the Ca^{2+}-dependent CAM, N-cadherin; and members of the integrin extracellular matrix receptor superfamily. Two other CAMs present on neurons and/or Schwann cells—N-CAM and myelin-associated glycoprotein—do not appear to be important in regulating process growth. Our results imply that neuronal growth cones use integrin-class extracellular matrix receptors and at least two CAMs—N-cadherin and L1/Ng-CAM—for growth on Schwann cells in vitro and establish each of these glycoproteins as a strong candidate for regulating axon growth and guidance in vivo.

A central question in developmental neuroscience concerns the mechanisms of axonal growth and guidance during embryogenesis, and the related mechanisms of axonal regeneration after injury. Both processes must involve recognition of surface and secreted molecules of nonneuronal cells by specific receptors on neuronal growth cones (Bentley and Caudy, 1983; Raper et al., 1984; McMahan et al., 1980). Experimental evidence suggests that, among vertebrate nonneuronal cells, Schwann cells are unique in their ability to stimulate growth and regeneration. Grafts of peripheral nervous tissue allow regeneration of central nervous system neurons, and it is likely that Schwann cells mediate this effect (Aguayo et al., 1987; Hall, 1986; Kromer and Cornbrooks, 1986). Other nonneuronal cell types, such as fibroblasts and astrocytes, seem unable to stimulate such regeneration (see Kromer and Cornbrooks, 1986). Schwann cells may also play an important role in axon growth during development. There is evidence that Schwann cells normally precede motor axons into chick limbs (Noakes and Bennett, 1987), and that their absence can lead to failure of the axons to invade the limb (Carpenter, E. M., and M. Hollyday, unpublished observations). The ability of Schwann cells to promote growth and regeneration persists in vitro, where they have been shown to be excellent substrates for neurite growth (Fallon, 1985; Tomaselli et al., 1986). What are the molecular interactions that underlie the unusual ability of Schwann cells to stimulate growth?

It has been clear for several years that macromolecules of the extracellular matrix (ECM)[1] can stimulate process outgrowth from a variety of neurons (e.g., Akers et al., 1981; Manthorpe et al., 1983; Rogers et al., 1983; Lander et al., 1982, 1985). These interactions are apparently mediated by neuronal surface proteins belonging to a family of adhesion receptor heterodimers known as integrins (Bozyczko and Horwitz, 1986; Tomaselli et al., 1986, 1987; Hynes, 1987; Ruoslahti and Pierschbacher, 1987). When neurons grow on cellular substrates, cell surface adhesion molecules (CAMs) are also involved in neurite promotion (Bixby et al., 1987; Chang et al., 1987; Tomaselli et al., 1988; Neugebauer et al., 1988). In previous experiments, we have used a population of peripheral motor neurons, chick ciliary ganglion (CG) neurons, to define specific molecular interactions responsible for neurite growth on the surfaces of several nonneuronal cell types. Our results with CG neuron growth on myotubes, astrocytes, and fibroblasts indicate that (*a*) neurons interact specifically with the Ca^{++}-dependent CAM, N-cadherin, and the Ca^{++}-independent CAM, N-CAM, as well as ECM macromolecules to produce neurites; and (*b*) the interactions that are important vary with the nonneuronal cell under consideration (Tomaselli et al., 1986, 1988; Bixby et al., 1987). In particular, CG neuron growth on myotubes involves interactions with ECM macromolecules as well as

John L. Bixby's present address is Department of Pharmacology, University of Miami School of Medicine, Miami, Florida 33136.

1. *Abbreviations used in this paper*: CAM, cell adhesion molecule; CG, ciliary ganglion; ECM, extracellular matrix; LN, laminin; MAG, myelin-associated glycoprotein.

N-cadherin and N-CAM, growth on astrocytes depends primarily on interactions involving N-cadherin and the ECM, and growth on fibroblasts depends largely on ECM interactions.

Schwann cells are known to produce an array of ECM molecules and CAMs including laminin (LN), collagen, N-cadherin, N-CAM, L1/Ng-CAM, cytotactin, and myelin-associated glycoprotein (MAG) (Cornbrooks et al., 1983; Carey et al., 1983; Hatta et al., 1985; Noble et al., 1985; Nieke and Schachner, 1985; Crossin et al., 1986; Sternberger et al., 1979). The experiments described in this report are directed towards defining which, if any, of these contribute to stimulation of process growth from peripheral motor neurons.

Materials and Methods

Animals

Fertile White Leghorn eggs were obtained from Feather Hill Farm (Petaluma, CA) and kept at 38°C and 95% humidity until use.

Chemicals and Reagents

Glial growth factor was partially purified (through the carboxymethyl-cellulose step) according to the procedures of Brockes et al. (1980). Protein A-Sepharose was from Pharmacia Fine Chemicals (Piscataway, NJ). DEAE-cellulose and carboxymethyl-cellulose were from Whatman Inc. (Clifton, NJ). Rabbit complement was from Gibco (Grand Island, NY). Forskolin was from Calbiochem-Behring Corp. (La Jolla, CA). All other chemicals were from Sigma Chemical Co. (St. Louis, MO).

Antibodies

Anti-N-cadherin serum was generated in rabbits against a 90-kD proteolytic fragment of an avian retinal Ca^{++}-dependent CAM (Crittenden et al., 1987). The serum recognizes a single 130-kD protein in one- and two-dimensional immunoblots of retina and brain (Crittenden et al., 1987). This 130-kD protein, previously named N-cal-CAM, is similar or identical to N-cadherin (Crittenden et al., 1988). The generation and specificity of the anti-N-CAM serum has been described previously (Bixby and Reichardt, 1987; Bixby et al., 1987). Rabbit antiserum to rat MAG was produced as described (Salzer et al., 1987) and was the generous gift of Dr. Jim Salzer (New York University, New York). Fab fragments of a rabbit antibody to mouse L1 were produced as described (Rathjen and Schachner, 1984) and were the generous gift of Dr. Fritz Rathjen (Max-Planck-Institute, Tübingen, FRG).

The hybridoma line secreting anti-integrin β_1 antibody (Neff et al., 1982; Buck et al., 1986) was the kind gift of Dr. Rick Horwitz (University of Illinois, Chicago, IL) and the hybridoma line secreting an anti-N-CAM antibody (224-1A6; Lemmon et al., 1982) was the kind gift of Dr. David Gottlieb (Washington University, St. Louis, MO). The hybridoma line secreting the A_2B_5 mAb was purchased from the American Type Culture Collection (Rockville, MD). Anti-Thy 1.1 IgG was purchased from New England Nuclear (Boston, MA).

224-1A6 IgG was purified from ascites fluid by ammonium sulfate precipitation and DEAE-cellulose chromatography as described in Hudson and Hay (1980). Anti-integrin β_1 IgG was purified on protein A-Sepharose as described in Neff et al. (1982). Anti-N-CAM, anti-N-cadherin, and anti-MAG IgGs were purified as described in Hudson and Hay (1980). Fab' fragments of anti-N-CAM and anti-N-cadherin IgG were prepared by pepsin digestion followed by reduction and alkylation as described in Brackenbury et al. (1977).

Cell Culture

Schwann cells were prepared from neonatal rat sciatic nerve (Brockes et al., 1979), and the first passage cells were the generous gift of Dr. Greg Lemke (Salk Institute, La Jolla, CA). These cells were >95% pure as judged by morphological criteria. Schwann cells were kept free of fibroblasts by treatment with anti-Thy 1.1 and rabbit complement. Briefly, cells were trypsinized, spun down, washed with Hepes-buffered DME/10% FCS, spun down, resuspended in 0.4 ml Hepes-buffered DME/10% FCS with a 1:100 dilution of anti-Thy 1.1 IgG, and incubated for 20 min at 37°C. Rabbit complement (diluted 1:6) was added and the cells were incubated for a further 40 min at 37°C. The cells were brought to 5 ml with Hepes-buffered DME/10% FCS, spun down, resuspended, and plated. Schwann cells were grown on poly-D-lysine-coated dishes in DME with 10% FCS, 100 U/ml penicillin/streptomycin, 2 μM forskolin, and 12 μg/ml crude glial growth factor. These conditions optimize growth of Schwann cells as opposed to fibroblasts (Lemke, G., unpublished observations; cf. Porter et al., 1986). For coculture with neurons, cells were passaged onto poly-D-lysine-coated glass coverslips and grown to confluence. CG neurons were prepared as described (Bixby and Reichardt, 1985) and plated onto the Schwann cell monolayers at a density of ~50/mm² in the CG medium. Antibodies were diluted in medium, filtered through 0.22-μm filters, and added to cultures at the same time as the neurons. Control cultures had medium diluted with PBS to the same extent as the most diluted antibody solution. CG neuron/chick embryo fibroblast cocultures and neuron/myotube cocultures were prepared as described (Bixby and Reichardt, 1985; Tomaselli et al., 1986).

Analysis of Neurite Outgrowth

Neuron/Schwann cell cocultures and neuron/myotube cocultures were fixed and stained with A_2B_5 antibody as described (Tomaselli et al., 1988). Neuron/fibroblast cocultures were fixed and stained as described (Tomaselli et al., 1986). Analysis of neurite outgrowth was performed as described (Tomaselli et al., 1988). Briefly, stained neurons (30 per condition) were traced with a drawing tube and a 63 × objective, and neurites were measured on a digitizing pad. For each neuron, the lengths of all neurites (>15 μm) were measured and these were added together to yield total neurite length. For each experimental condition, the neurite length data were combined with data on the percentage of neurons bearing neurites (>15 μm) to yield the final distribution curves. A neurite shorter than 15 μm was not measured; neurons with no neurites longer than 15 μm were scored as negative for neurites. Distribution curves are plotted for individual experiments in Figs. 2 and 3; there is some variation in control length from experiment to experiment.

Results

CG neurons appear to extend processes far more rapidly on Schwann cells than on any other substrate examined. (Table I). When neurons were plated on Schwann cells and examined after 6–7 h, the total neuritic output of a neuron, on average, was 550 μm (Table I), corresponding to an average rate of growth of 75 μm/h. CG neurons grew at ~50% of this rate on chick myotubes (35 μm/h). The growth rate we measured on myotubes is essentially the same as that found by Role and Fischbach (1987). These workers found a linear growth rate of 40 μm/h for the first 72 h in culture, which suggests that the apparent growth rates we have determined with single

Table I. Rate of Neurite Growth by CG Neurons on Various Substrates

Substrate	Mean neurite length	Time	Growth rate
	μm	h	μm/h
Schwann cells	550	6–7	75 ± 6
Myotubes	242	7	35
		1–72	40‡
Astrocytes*	300	16–20	18 ± 0.7
Fibroblasts	150	14	11
LN*	210	16–18	13 ± 2

Apparent growth rates were derived by dividing mean total neurite length by time in culture. The data from Role and Fischbach (1987) are from a graph of neurite length vs. time in culture; the rate was invariable for 3 d. The Schwann cell data are from six separate control determinations, the astrocyte data from four determinations, and the LN data from four determinations; the growth rates are the means ± SEMs of these.

* Data from experiments in Tomaselli et al., 1988.

‡ Data from Role and Fischbach, 1987.

time points are valid. Table I also shows the apparent rate of growth of CG neurons on astrocytes and fibroblasts, as well as on LN, the most potent inducer of outgrowth among known ECM molecules (cf. Manthorpe et al., 1984; Rogers et al., 1983; Hall et al., 1987). All of these growth rates are considerably lower than that on myotubes, but growth on astrocytes (~18 μm/h) is significantly faster than on fibroblasts or purified LN (11–13 μm/h; $p < 0.05$, t test).

The very high rate of growth of CG neurons on Schwann cells compared with other cell types suggests that CG neurons interact with Schwann cells in a unique fashion, possibly involving a molecular interaction not previously defined for other substrates. To identify the molecules mediating the interactions between CG neurons and Schwann cells that result in process outgrowth, neurons were grown on a monolayer of Schwann cells in the absence or presence of different antibodies. Since Schwann cells synthesize and accumulate on their surfaces several ECM proteins, including LN and collagen IV (Cornbrooks et al., 1983; Carey et al., 1983), it seemed likely that these would contribute to process outgrowth. Indeed, ECM molecules deposited by Schwann cells into a detergent-insoluble fraction have previously been shown to promote vigorous neurite outgrowth (Tomaselli et al., 1986). Therefore, antibodies to the major neuronal receptors for these ECM glycoproteins—the integrins—were tested for inhibitory effects on neurite growth. The β_1 subunit of the integrins has been shown to associate with at least five different α subunits in heterodimers that function as receptors for LN, fibronectin, and several collagens (reviewed in Ruoslahti and Pierschbacher, 1987). Antibodies to the β_1 subunit have been shown to virtually eliminate neuronal attachment to these ECM glycoproteins and to prevent neuronal process outgrowth on substrates coated with these proteins, intact ECMs, or the detergent-insoluble exudate produced by Schwann cells (Boczyczko and Horwitz, 1986; Tomaselli et al., 1986; Hall et al., 1987). When tested alone, however, these antibodies had no detectable effect on the extent of neurite outgrowth by CG neurons on intact Schwann cells (Figs. 1, 3, and 4 *A*, *b*). Since contact with Schwann cell surfaces can promote integrin-independent neurite outgrowth (Tomaselli et al., 1986), these results suggest that molecules associated with the Schwann cell plasmalemma are likely to be more important than secreted ECM glycoproteins in promoting process outgrowth on intact Schwann cells.

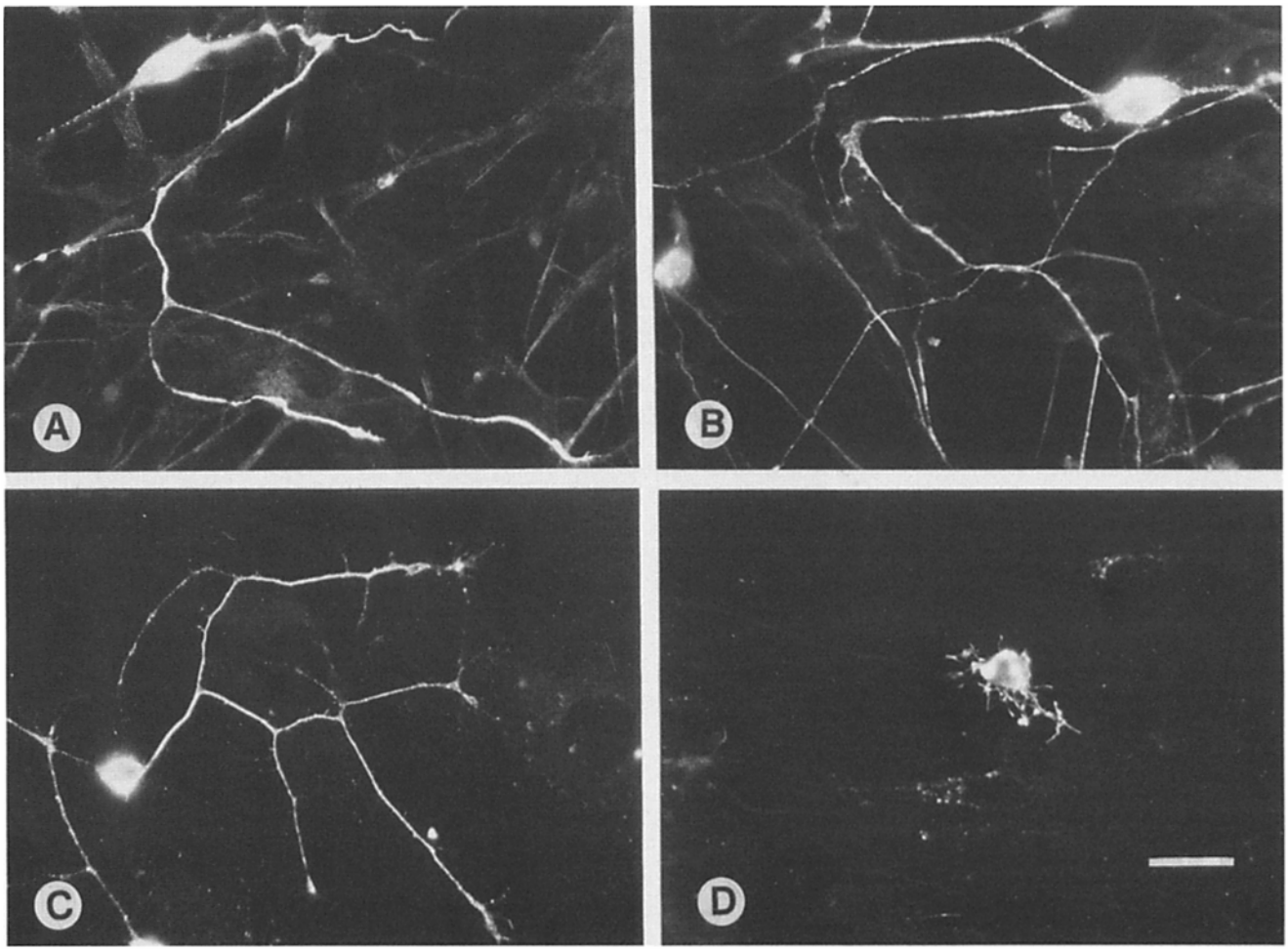

Figure 1. Immunofluorescence photographs of CG neurons grown on Schwann cell monolayers. Neurons were grown for 6–7 h, then fixed, and stained with A_2B_5. (*A*) Control. (*B*) Neurons grown in the presence of anti-integrin IgG (100 μg/ml) and anti-N-CAM IgG (1.3 mg/ml). (*C*) Neurons grown in the presence of anti-integrin IgG (100 μg/ml) and anti-L1-Fab (1 mg/ml). (*D*) Neuron grown in the presence of anti-integrin IgG (100 μg/ml), anti-L1-Fab (1 mg/ml), and anti-N-cadherin IgG (1 mg/ml). Bar, 10 μm.

As several different CAMs have been shown to promote neuronal process outgrowth on other cells, including N-cadherin on astrocytes and skeletal myotubes, N-CAM on skeletal myotubes, and L1/Ng-CAM on sympathetic axons (Bixby et al., 1987; Chang et al., 1987; Tomaselli et al., 1988), antibodies and Fab fragments to those CAMs which have been reported to be on Schwann cells—N-CAM, MAG, L1/Ng-CAM, and N-cadherin—were tested for inhibitory effects on Schwann cell-dependent neurite outgrowth.

The cadherins are a family of Ca^{2+}-dependent CAMs that are widely distributed in developing embryos and adult animals (Hatta et al., 1985, 1987, 1988). N-cadherin has been shown previously to be one of three important glycoproteins that promote CG neuron process growth on skeletal myotubes and to be the major protein that promotes process growth by the same neurons on astrocytes (Bixby et al., 1987; Tomaselli et al., 1988). When tested for effects on CG neuron process outgrowth on Schwann cells, anti-N-cadherin IgG inhibited growth significantly. When added at a saturating concentration (1 mg/ml; see Bixby et al., 1987; Tomaselli et al., 1988), anti-N-cadherin IgG reduced neuritic output by ~35% (Figs. 2 and 4 *A*, *f*). Control experiments showed that anti-N-cadherin IgG does not inhibit process growth by CG neurons on LN substrates (Fig. 4 *B*; Tomaselli et al., 1988). Previous results have shown that this antibody also does not inhibit neurite outgrowth on fibroblasts (Bixby et al., 1987). Therefore, the present results indicate that N-cadherin function is involved in CG neuron growth on Schwann cells.

The Ca^{2+}-independent CAM, L1/Ng-CAM, has been shown to be present on both neurons and Schwann cells in developing peripheral nerve (Rathjen and Schachner, 1984; Grumet et al., 1984; Faissner et al., 1984; Nieke and Schachner, 1985; Noble et al., 1985). This CAM appears to be the same as two independently identified chick antigens named G4 and 8D9 (Rathjen et al., 1987; Lemmon and McCloon, 1986), which have been shown, respectively, to contribute to outgrowth of sympathetic neurons on sympathetic axons (Chang et al., 1987) and to promote neurite outgrowth by several different classes of neurons when purified and immobilized on a substrate (Lagenauer and Lemmon, 1987).

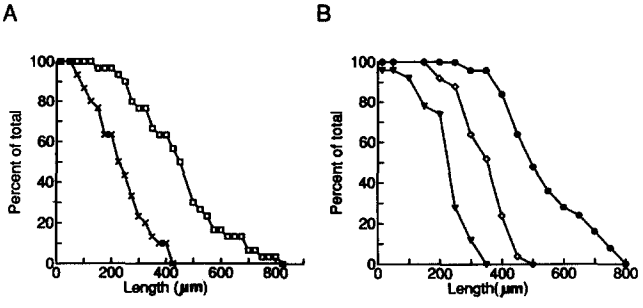

Figure 2. Distribution of neurite lengths of CG neurons grown on Schwann cell surfaces. The total neurite length of individual neurons is plotted against the percentage of neurons in the culture exceeding that length. (*A*) Neurons were cultured for 8 h in the presence of anti-integrin IgG at 100 μg/ml (*open squares*) or anti-N-cadherin IgG at 1 mg/ml (*crosses*). (*B*) Neurons were cultured for 7 h with no antibody (*solid circles*), anti-L1 Fab at 1 mg/ml (*open diamonds*), or anti-L1 Fab plus anti-N-cadherin Fab (*inverted solid triangles*).

The contribution of L1/Ng-CAM to process growth was tested by culturing CG neurons and Schwann cells in the presence of anti-L1 Fab fragments. The presence of this antibody reduced neurite growth by ~35% (Figs. 2 *B* and 4 *A*, *g*). The concentration used, 1 mg/ml, has been shown to be saturating (Rathjen and Schachner, 1984). As has been demonstrated for the other anti-CAM IgGs we have used (Bixby et al., 1987; Tomaselli et al., 1988), the inhibition of process growth by anti-L1 Fab was not due to a nonspecific toxic or growth inhibitory effect, since anti-L1 Fab had no effect on CG neuron growth on LN substrates (Fig. 4 *B*). Therefore, the results establish the importance of the Ca^{++}-independent CAM L1/Ng-CAM as a mediator of neuronal process growth on Schwann cells in vitro.

Since both L1/Ng-CAM and N-cadherin are able to contribute to CG neuron process growth on Schwann cells, we examined the effect of the combination of antibodies to these molecules. When added together, anti-N-cadherin Fab fragments and anti-L1 Fab fragments had a strong inhibitory effect, reducing process outgrowth by almost 60% (Figs. 2 *B* and 4 *A*, *j*). Two points emerge from these data. (*a*) Interactions mediated by these two CAMs account for a large proportion of the total neurite growth by CG neurons on Schwann cells. (*b*) Important contributions must be made by other, as yet unidentified, interactions.

As we had previously shown that ECM molecules secreted by Schwann cells can stimulate CG neuron process growth in an integrin-dependent manner (Tomaselli et al., 1986), we tested the effects of anti-integrin β_1 antibodies in combination with either anti-L1 Fab, anti-N-cadherin Fab, or anti-N-cadherin IgG. Combinations of anti-integrin with Fab fragments of either CAM reduced neurite growth by ~50%, and the combination with anti-N-cadherin IgG was even more effective, reducing growth by ~60% (Figs. 3 and 4 *A*). Direct comparisons of the effects of these combinations to the effects of the corresponding CAM antibodies alone (Fig. 4, compare *A*, *f* with *A*, *k*; and *A*, *g* with *A*, *i*) show that integrin β_1 antibodies reduce process growth consistently when tested in combination. This implies that ECM interactions with integrin receptors do contribute to CG neuron process growth on intact Schwann cells, despite the failure of anti-integrin antibodies alone to inhibit growth significantly. As we have found previously (Bixby et al., 1987; Tomaselli et al., 1988), the inhibition produced by anti-N-cadherin was not due to cross-linking of the antigens, since Fab' fragments of anti-N-cadherin IgG were nearly as effective (Figs. 3 *B* and 4, *A*, *h*). The effects of anti-integrin β_1 have previously been shown not to depend on cross-linking (Tomaselli et al., 1986, 1988). As a control for the specificity of the anti-integrin β_1 IgG, it has been shown previously that this antibody preparation does not affect process growth of embryonic day 14 CG neurons on astrocytes (Tomaselli et al., 1988). Thus, the effects of L1/Ng-CAM, N-cadherin, and integrin β_1 antibodies are substrate specific and are observed using monovalent Fab fragments.

The combination of all three inhibitory antibodies—anti-N-cadherin IgG, anti-L1-Fab, and anti-integrin IgG—reduced CG neuron growth on Schwann cells by ~85%; ~50% of the neurons grew no neurites in the 7-h test period (Figs. 3 *B* and 4 *A*, *l*). The effects of these antibodies were on growth and not simply adhesion, since the neurons adhered to Schwann cells in similar numbers in these and control cul-

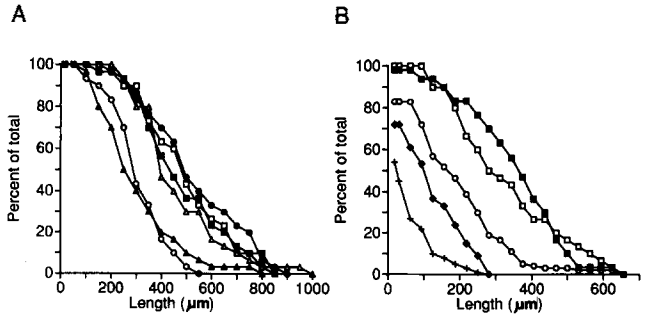

Figure 3. Distribution of neurite lengths of CG neurons grown on Schwann cell surfaces, plotted as in Fig. 2. Neurons were cultured for 6 h in the presence or absence of various antibodies. (*A*) Neurons cultured with no antibodies (*solid circles*), anti-integrin IgG at 100 μg/ml (*open squares*), anti-integrin IgG plus anti-N-CAM IgG at 1.2 mg/ml (*open triangles*), anti-integrin IgG plus anti-MAG IgG at 1 mg/ml (*solid squares*), anti-integrin IgG plus anti-N-cadherin Fab at 1 mg/ml (*open circles*), or anti-integrin IgG plus anti-L1 Fab at 1 mg/ml (*solid triangles*). (*B*) Neurons were grown with anti-integrin IgG (*open squares*), anti-integrin IgG plus anti-MAG IgG (*solid squares*), anti-integrin IgG plus anti-N-cadherin Fab (*open circles*), anti-integrin IgG plus anti-N-cadherin IgG (*solid diamonds*), or anti-integrin IgG, anti-N-cadherin IgG, and anti-L1-Fab (*plus signs*). The concentrations of all antibodies were the same as in *A*.

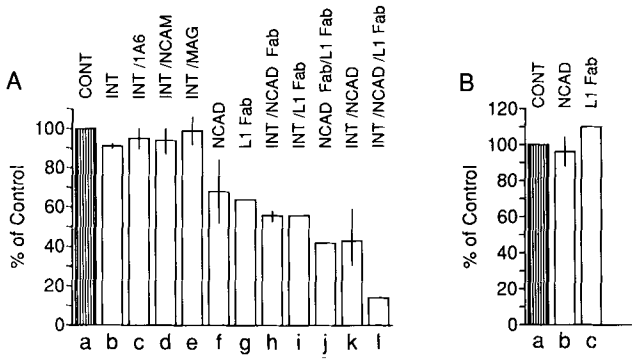

Figure 4. (*A*) Average neurite lengths of neurons grown on Schwann cells in the presence or absence of various antibodies. The control bar (*CONT*, striped bar) is 100% in all experiments by definition. Antibodies used in other experiments are as follows: *INT*, anti-integrin β_1 subunit IgG; *1A6*, monoclonal anti-N-CAM IgG; *NCAM*, anti-N-CAM IgG; *MAG*, anti-MAG IgG; *NCAD*, anti-N-cadherin IgG; *L1 Fab*, anti-L1 Fab; *NCAD Fab*, anti-N-cadherin Fab. Concentrations of antibodies were the same as in Fig. 3, and 1A6 IgG was used at 500 μg/ml. (*B*) Neurite lengths of neurons grown on LN substrates. Abbreviations of antibodies and their concentrations were the same as in *A*. Data in *B* are from Tomaselli et al., 1988. Data from different experiments were pooled by expressing each average as a percentage of the control average for that experiment. Vertical lines indicate the range of multiple experiments. Experiments in *A*, *f-l*, were all significantly different from controls. No other individual determinations differed significantly from controls (tests for comparing percentages; Bailey, 1981).

tures, and even extended lamellipodia or short neurites. These results suggest that most neurite growth by CG neurons on Schwann cells is attributable to interactions involving N-cadherin, L1/Ng-CAM, and the integrin β_1 family of ECM receptors.

The Ca^{++}-independent CAM, N-CAM, has been implicated in a variety of cell recognition events during development (Edelman, 1985), and participates in CG neuronal fasciculation and process outgrowth on myotubes (Bixby and Reichardt, 1987; Bixby et al., 1987). We therefore tested antibodies to N-CAM for effects on process growth on Schwann cells. Two different antibodies to N-CAM were used in these experiments. One, the mAb 224-1A6-A1 (hereafter referred to as 1A6), does not perturb N-CAM function as assayed by vesicle aggregation or neurite outgrowth (Watanabe et al., 1986; Bixby et al., 1987) even though it binds well to CG neurons (Bixby et al., 1987). The second is an N-CAM-function blocking polyclonal IgG preparation (Bixby et al., 1987; Bixby and Reichardt, 1987). Neither IgG detectably reduced CG neuron process extension on Schwann cells, either alone (not shown) or in combination with anti-integrin β_1 IgG (Figs. 1, 3, and 4 *A*, *c* and *d*). Fab fragments of 1A6 were not tested; the polyclonal anti-N-CAM Fabs also had no detectable effect (not shown). The results argue that N-CAM is not important for process outgrowth by CG neurons on Schwann cells. In contrast, N-CAM may be involved in dorsal root ganglion neuron outgrowth on Schwann cells (Seilheimer and Schachner, 1988).

MAG is present on the surfaces of Schwann cells in developing peripheral nerve in vivo and many neurons in vitro (Sternberger et al., 1979; Omlin et al., 1985; Martini and Schachner, 1986), and may function in neuron-oligodendrocyte adhesion and myelination (Quarles, 1984; Poltorak et al., 1987). MAG, a member of the immunogobulin superfamily, is structurally related to N-CAM (Salzer et al., 1987) and has recently been shown to function, like N-CAM, as a Ca^{2+}-independent CAM (Poltorak et al., 1987). Therefore, anti-MAG IgG was tested for effects on CG neuron process outgrowth, in combination with anti-integrin β_1 IgG. No effects on neurite growth were seen (Figs. 3 and 4 *A*, *e*). These results suggest that MAG is not important in promoting process growth on Schwann cells in vitro. As MAG is expressed on Schwann cells at higher levels in vivo than in vitro (Martini and Schachner, 1986; Poltorak et al., 1987), it remains possible that it may serve such a role in vivo.

In summary, antibodies to two CAMs, N-cadherin and L1-Ng/CAM, had major inhibitory effects on the profuse neurite outgrowth induced by Schwann cells. Anti-integrin β_1 ECM receptor antibodies, which bind to CG neurons and inhibit neuronal interactions with ECM macromolecules, had more subtle effects, detectable when other interactions were diminished. Finally, antibodies to other CAMs present on CG neurons and/or Schwann cells (N-CAM and MAG) had no detectable effects on neurite growth.

Discussion

The major conclusion of this paper is that peripheral motor neurons use three major interactions for process outgrowth on Schwann cells in vitro. As process outgrowth was reduced to <15% of controls by the inhibition of interactions between integrins and the ECM, N-cadherin and its ligand, and L1/Ng-CAM and its receptor, these appear to be the major interactions through which Schwann cells promote neurite growth in vitro (Fig. 5). The residual neurite growth in these conditions could be due to incomplete block by the antibodies (see below) or to the presence of additional interactions.

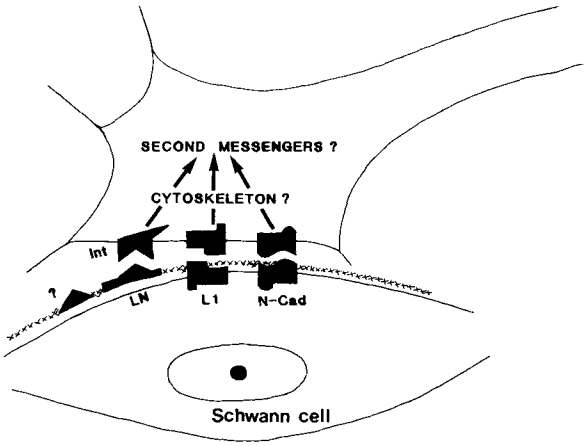

Figure 5. Model of surface interactions mediating neuronal process growth on Schwann cells. The model illustrates a neuronal growth cone (*above*) interacting with a Schwann cell (*below*). The model shows L1/Ng-CAM (*L1*) and N-cadherin (*N-Cad*) in the Schwann cell membrane interacting by homophilic binding with the same molecules in the growth cone membrane. Similarly, ECM proteins secreted by Schwann cells, including laminin (*LN*) and additional, unidentified proteins (*?*) are shown interacting with ECM receptors of the integrin family (*Int*) in the growth cone membrane. All three binding events are shown leading to intracellular changes in the growth cone through the interaction of intracellular domains with hypothetical cytoskeletal elements and "second messengers." These, in turn, are postulated to lead to axonal elongation.

A second conclusion is that differences in the rate of neuronal process outgrowth stimulated by different nonneuronal cell types correlate with differences in the molecular interactions underlying this growth. For example, the rates of CG neuronal growth on LN and on fibroblasts (largely due to ECM molecules) are roughly equivalent. Myotubes, astrocytes, and Schwann cells all stimulate growth through ECM molecules as well as various CAMs, and rates of growth on these cells are higher. Differences among these three cell types in their ability to stimulate CG neuron growth probably reflect both quantitative differences in expression of these molecules (e.g., higher levels of N-CAM on myotubes compared to Schwann cells or astrocytes) and qualitative differences in the abilities of the different CAMs and integrins to promote growth. It will be necessary to measure surface levels of ECM molecules, CAMs, and their respective neuronal receptors to determine the relative contributions of such quantitative and qualitative differences.

L1/Ng-CAM antibodies have also been shown to inhibit process outgrowth by mouse sensory neurons on mouse Schwann cells (Seilheimer and Schachner, 1988). Thus, L1/Ng-CAM seems likely to mediate process outgrowth by all the classes of neurons whose axons are localized in peripheral nerve. In contrast to our results using chick CG neurons, L1/Ng-CAM antibodies alone had dramatic inhibitory effects on process outgrowth by murine sensory neurons (75–88% reduction). These quantitative differences could reflect more efficient recognition by antibodies of the L1/Ng-CAM on the murine neurons or differences between sensory and CG neurons in the interactions used for process outgrowth. As integrins and N-cadherin are present on sensory neurons (Bozyczko and Horwitz, 1986; Hatta et al., 1987), the strong inhibitory effects of L1/Ng-CAM antibodies on sensory neuron process outgrowth may reflect, in part, interactions between different classes of adhesion molecules. Consistent with this possibility, L1/Ng-CAM antibodies were also observed to inhibit process outgrowth by murine sensory neurons on collagen substrates (Schachner, M., unpublished observations).

The molecular interactions we have defined each consist of an adhesive molecule on the substrate Schwann cell being recognized by a receptor in the neuronal membrane (Fig. 5). For interactions involving ECM macromolecules produced by nonneuronal cells, it seems that the neuronal receptors involved are members of the integrin β_1 ECM receptor family (Tomaselli et al., 1986, 1987; Bozyczko and Horwitz, 1986; cf. Hynes, 1987; Ruoslahti and Pierschbacher, 1987). It is likely that the major neurite-promoting ECM protein on Schwann cells is LN, as antibodies to an LN–heparan sulfate proteoglycan complex have been shown to strongly inhibit process growth by CG neurons on the detergent-insoluble, ECM-containing exudates secreted by Schwann cells in vitro (Tomaselli et al., 1986). Although receptor interactions with N-cadherin are less well studied, there is also strong evidence that it binds to itself, at least in part, in a "homophilic" interaction (Hatta et al., 1988). The CG neuronal receptor for N-cadherin, then, may be N-cadherin itself, similar to the situation postulated for N-CAM–mediated interactions (Edelman, 1985). Recent work has shown that the L1/Ng-CAM glycoprotein also binds to itself in a homophilic interaction; i.e., the major neuronal receptor for L1/Ng-CAM is itself (Grumet and Edelman, 1988). These workers also observed that binding of L1/Ng-CAM to avian brain astroglia seems to involve a receptor on astroglia distinct from L1/Ng-CAM. As L1/Ng-CAM is present on both the Schwann cells and neurons used in our experiments, homophilic interactions seem likely to be responsible for L1/Ng-CAM-dependent neurite outgrowth on Schwann cells. However, it is possible that heterophilic binding is also important. If so, heterophilic interactions of the avian L1/Ng-CAM with a possible receptor on Schwann cells could account for the residual neurite outgrowth seen in the presence of the three antibodies, as the L1/Ng-CAM antibodies used bind only weakly to the avian molecule (Rathjen and Schachner, 1984).

Regardless of the details of molecular interactions among the CAMs, the major finding of this paper and our previous work is that both integrin-related neuronal ECM receptors and bona fide neuronal CAMs can function as receptors mediating neurite growth. Thus, substrate-adhesion molecules and CAMs can serve complementary functions in promoting neuronal process outgrowth despite their very different structures.

Similar to our previous findings on other cellular substrates (Tomaselli et al., 1986, 1988; Bixby et al., 1987), neurons were adherent to their Schwann cell substrate, with lamellipodial extensions, even in conditions which severely inhibited neurite growth. These results, like those of others (Gunderson, 1987; Hall et al., 1987), indicate that extension of neurites is promoted by a subset of the interactions that promote substrate adhesion. It is likely, therefore, that some adhesive interactions of neurons with Schwann cells (e.g., those mediated by MAG) may have functions that do not include induction of neurite outgrowth. As N-CAM has been

shown to be involved in process growth by CG neurons growing on the surfaces of skeletal myotubes, the failure of N-CAM antibodies to inhibit process outgrowth by the same neurons on Schwann cells must reflect differences in either the amount or forms of N-CAM present on myotubes as compared to Schwann cells.

It seems likely, then, that integrins, N-cadherin, and L1/Ng-CAM are not simply "glue" but stimulate neurite production through intracellular actions via their cytoplasmic domains. There is strong evidence that both integrins and CAMs can bind to cytoskeletal elements (cf. Horwitz et al., 1986; Geiger et al., 1985; Volk et al., 1987; Pollerberg et al., 1986; Hirano et al., 1987). Whether cytoskeletal interactions of these molecules are necessary for neurite extension remains to be established. In platelets, there is evidence that the binding of integrins to their ligands induces changes in intracellular "second messengers" (Banga et al., 1986). If the integrins and CAMs involved in neurite growth were to act through a common second messenger, it could explain the apparent additivity of interactions involving the different adhesion molecules (Fig. 5).

The substrate interactions that promote neurite outgrowth on Schwann cells in vitro are likely to be important in regulating axon growth in vivo, both during development, where there is now evidence that Schwann cells may help promote outgrowth by pioneering motor axons (Noakes and Bennett, 1987; Carpenter, E. M., and M. Hollyday, unpublished observations) and in regeneration. Schwann cells are a prominent cellular constituent of the peripheral nerve substrates contacted by regenerating axons (Aguayo et al., 1987). Expression of these neurite outgrowth-promoting glycoproteins may therefore account for some of the differences in the regenerative potential of peripheral vs. central nerves. As shown in this paper, Schwann cells are by far the best substrate for promoting neurite outgrowth by CG neurons in vitro. Similarly, segments of peripheral nerve containing living Schwann cells are very effective at promoting regeneration in vivo, even of central nervous system neurons that normally do not regenerate (Kromer and Cornbrooks, 1986; So and Aguayo, 1985). In contrast, the central nervous system does not support regeneration and the major non-neuronal cell types present—astrocytes and oligodendrocytes—are much less effective than Schwann cells at promoting neurite outgrowth in vitro. Thus, astrocytes were shown to be fourfold less efficient than Schwann cells at stimulating process outgrowth by CG neurons in vitro. Oligodendrocytes have been shown to contain surface proteins that actually inhibit axon growth in vitro (Caroni and Schwab, 1988).

Furthermore, regulation of these adhesive glycoproteins on both neurons and their cellular substrates may determine the extent of reinnervation after peripheral and central nerve injury. There is direct evidence that the functions of integrin-class ECM receptors are regulated during development. In particular, older neurons lose responsiveness to many ECM glycoproteins (Cohen et al., 1986, 1987; Hall et al., 1987; Tomaselli et al., 1988) and the loss of responsiveness may be regulated by target contact (Collins and Lee, 1982; Cohen, J., unpublished observations). Expression of L1/Ng-CAM has also been shown to be developmentally regulated in neuronal and glial cells (Martini and Schachner, 1986) although it is not clear whether L1/Ng-CAM and/or N-cadherin are induced in neurons after denervation. Finally, L1/Ng-CAM is clearly induced in Schwann cells and fibroblasts in injured peripheral nerve (Nieke and Schachner, 1985; Daniloff et al., 1986). The regulation of N-cadherin has not yet been studied in this system.

While differences in surface-bound adhesive glycoproteins may account for much of the difference between regeneration in peripheral and central nerves, it is also clear that the cells in denervated peripheral nerves are induced to make soluble trophic molecules, most notably nerve growth factor, by lymphokines (e.g., IL-1) secreted by invading macrophages (Heumann et al., 1987; Lindholm et al., 1987). Thus, trophic factors may also function as inducers of axonal regeneration in denervated peripheral nerves, and it will be important to determine whether the same lymphokines that regulate expression of nerve growth factor also regulate expression of N-cadherin, L1/Ng-CAM, and the integrins.

In conclusion, regulation of CAMs and integrins seems likely to be important in determining the rate and extent of axon growth during development and in regeneration. A more detailed understanding of the factors that regulate their expression could be a major advance in clinical approaches to nerve injury.

We thank Fritz Rathjen, Rick Horwitz, David Gottlieb, and Jim Salzer for their generous gifts of antibodies; Greg Lemke for his kind gift of Schwann cells and his good advice concerning their care and feeding; Drs. Bernd Seilheimer and Melitta Schachner for sharing their results with us before publication; Kevin Tomaselli for discussion of unpublished data; Steve Lisberger for the use of his graphics program (which produced most of the figures); Karla Neugebauer for purification of CSAT IgG; Kathy Buckley, Zach Hall, Kevin Tomaselli, and Reg Kelly for their cogent comments on the manuscript; and Marion Meyerson for her excellent typing.

This research was supported by National Institutes of Health grant NS19090 and the Howard Hughes Medical Institute. L. F. Reichardt is an Investigator of the Howard Hughes Medical Institute.

Received for publication 11 January 1988, and in revised form 3 March 1988.

References

Aguayo, A. J., M. Vidal-Sanz, M. P. Villegas-Perez, and G. M. Bray. 1987. Growth and conductivity of axotomized retinal neurons in adult rats with optic nerves substituted by peripheral nervous system grafts—linking the eye and the midbrain. *Ann. N.Y. Acad. Sci.* 495:1-9.

Akers, R. M., D. F. Mosher, and J. E. Lilien. 1981. Promotion of retinal neurite outgrowth by substratum bound fibronectin. *Dev. Biol.* 86:179-188.

Balley, N. T. 1981. Statistical Methods in Biology. 2nd ed. Hotter and Stodin, London. pp. 1-216.

Banga, H., E. R. Simons, L. F. Brass, and S. E. Rittenhouse. 1986. Activation of phospholipases A and C in human platelets exposed to epinephrine: role of glycoproteins IIb/IIIa and dual role of epinephrine. *Proc. Natl. Acad. Sci. USA.* 83:9197-9201.

Bentley, D., and M. Caudy. 1986. Pioneer axons lose directed growth after selective killing of guidepost cells. *Nature (Lond.).* 304:62-65.

Bixby, J. L., and L. F. Reichardt. 1985. The expression and localization of synaptic vesicle antigens at neuromuscular junctions in vitro. *J. Neurosci.* 5: 3070-3080.

Bixby, J. L., and L. F. Reichardt. 1987. Effects of antibodies to N-CAM on the differentiation of neuromuscular junctions in vitro. *Dev. Biol.* 119:363-372.

Bixby, J. L., R. S. Pratt, J. Lilien, and L. F. Reichardt. 1987. Neurite outgrowth on muscle cell surfaces involves extracellular matrix receptors as well as Ca^{++}-dependent and independent cell adhesion molecules. *Proc. Natl. Acad. Sci. USA.* 84:2555-2559.

Bozyczko, D., and A. F. Horwitz. 1986. The participation of a putative cell surface receptor for laminin and fibronectin in peripheral neurite extension. *J. Neurosci.* 6:1241-1251.

Brackenbury, R., J. P. Thiery, U. Rutishauser, and G. M. Edelman. 1977. Adhesion among neural cells of the chick retina. I. An immunological assay for molecules involved in cell-cell binding. *J. Biol. Chem.* 252:6835-6840.

Brockes, J. P., K. L. Fields, and M. C. Raff. 1979. Studies on cultured rat Schwann cells. I. Establishment of purified populations from cultures of pe-

ripheral nerve. *Brain Res.* 165:105–118.
Brockes, J. P., G. E. Lemke, and D. R. Balzer. 1980. Purification and preliminary characterization of a glial growth factor from the bovine pituitary. *J. Biol. Chem.* 255:8374–8377.
Buck, C. A., E. Shea, K. Duggan, and A. F. Horwitz. 1986. Integrin (the CSAT antigen): functionality requires oligomeric integrity. *J. Cell Biol.* 103:2421–2428.
Carey, D. J., C. F. Eldridge, C. J. Cornbrooks, R. Timpl, and R. P. Bunge. 1983. Biosynthesis of type IV collagen by cultured rat Schwann cells. *J. Cell Biol.* 97:473–479.
Caroni, P., and E. Schwab. 1988. Antibody against myelin-derived, membrane-bound inhibitor of neurite growth neutralizes CNS white matter non-permissive substrate properties. *Neuron.* 1:85–96.
Chang, S., F. G. Rathjen, and J. A. Raper. 1987. Extension of neurites on axons is impaired by antibodies against specific neural cell surface glycoproteins. *J. Cell Biol.* 104:355–362.
Cohen, J., J. F. Burne, C. McKinley, and J. Winter. 1987. The role of laminin and the laminin/fibronectin receptor complex in the outgrowth of retinal ganglion cell axons. *Dev. Biol.* 122:407–418.
Cohen, J., J. F. Burne, J. Winter, and P. Bartlett. 1986. Retinal ganglion cells lose response to laminin with maturation. *Nature (Lond.).* 322:465–467.
Collins, F., and M. R. Lee. 1982. A reversible developmental change in the ability of ciliary ganglion neurons to extend neurites in culture. *J. Neurosci.* 2:424–430.
Cornbrooks, C. J., D. J. Carey, J. A. McDonald, R. Timpl, and R. P. Bunge. 1983. In vivo and in vitro observations on laminin production by Schwann cells. *Proc. Natl. Acad. Sci. USA.* 80:3850–3854.
Crittenden, S. L., R. S. Pratt, J. H. Cook, J. Balsamo, and J. Lilien. 1987. Immunologically unique and common domains within a family of proteins related to the retinal Ca^{++}-dependent cell adhesion molecule, NcalCAM. *Development (Camb.).* 101:729–740.
Crittenden, S. L., U. Rutishauser, and J. Lilien. 1988. Identification of two structural types of Ca^{++}-dependent cell adhesion molecules in the chick. *Proc. Natl. Acad. Sci. USA.* 85:3464–3468.
Crossin, K. L., S. Hoffman, M. Grumet, J.-P. Thiery, and G. Edelman. 1986. Site-restricted expression of cytotactin during development of the chick embryo. *J. Cell Biol.* 102:1917–1930.
Daniloff, J. K., G. Levi, M. Grumet, F. Rieger, and G. M. Edelman. 1986. Altered expression of neuronal cell adhesion molecules induced by nerve injury and repair. *J. Cell Biol.* 103:929–945.
Edelman, G. M. 1985. Cell adhesion and the molecular processes of morphogenesis. *Annu. Rev. Biochem.* 54:135–169.
Faissner, A., J. Kruse, J. Nieke, and M. Schachner. 1984. Expression of neural cell adhesion molecule L1 during development, in neurological mutants, and in the peripheral nervous system. *Dev. Brain Res.* 15:69–82.
Fallon, J. R. 1985. Neurite guidance by non-neuronal cells in culture: preferential outgrowth of peripheral neurites on glial as compared to non-glial cell surfaces. *J. Neurosci.* 5:3169–3177.
Geiger, B., Z. Asvnur, T. Volberg, and T. Volk. 1985. Molecular domains of adherens junctions. *In* The Cell in Contact. G. M. Edelman and J.-P. Thiery, editors. Neuroscience Institute, New York. 461–490.
Grumet, M., and G. M. Edelman. 1988. Neuron-glia cell adhesion molecule interacts with neurons and astroglia via different binding mechanisms. *J. Cell Biol.* 106:487–504.
Grumet, M., S. Hoffman, and G. Edelman. 1984. Two antigenically related neuronal cell adhesion molecules of different specificities mediate neuron-neuron and neuron-glia adhesion. *Proc. Natl. Acad. Sci. USA.* 81:267–271.
Gunderson, R. W. 1987. Response of sensory neurites and growth cones to patterned substrate of laminin and fibronectin in vitro. *Dev. Biol.* 121:423–431.
Hall, D. E., K. M. Neugebauer, and L. F. Reichardt. 1987. Embryonic neural retinal cell response to extracellular matrix proteins: developmental changes and effects of the CSAT antibody. *J. Cell Biol.* 104:623–634.
Hall, S. M. 1986. The effects of inhibitors of Schwann cell mitosis on the reinnervation of acellular autografts in the peripheral nervous system of the mouse. *Neuropathol. Appl. Neurobiol.* 12:401–414.
Hatta, K., A. Nose, A. Nagafuchi, and M. Takeichi. 1988. Cloning and expression of cDNA encoding neural calcium-dependent cell adhesion molecule: its identity in the cadherin gene family. *J. Cell Biol.* 106:873–881.
Hatta, K., T. S. Okada, and M. Takeichi. 1985. A monoclonal antibody disrupting calcium-dependent cell–cell adhesion of brain tissues. Possible role of its target antigen in animal pattern formation. *Proc. Natl. Acad. Sci. USA.* 82:2789–2793.
Hatta, K., F. Takaga, H. Fujisawa, M. Takeichi. 1987. Spatial and temporal expression pattern of N-cadherin cell adhesion molecule correlated with morphogenetic processes of chicken embryos. *Dev. Biol.* 120:215–227.
Heumann, R., D. Lindholm, C. Bandtlow, M. Meyer, M. J. Radeke, T. P. Misko, E. Shooter, and H. Thoenen. 1987. Differential regulation of nerve growth factor (NGF) and NGF-receptor mRNA in the rat sciatic nerve during development, degeneration, and regeneration: role played by macrophage. *Proc. Natl. Acad. Sci. USA.* 84:8735–8739.
Hirano, S., A. Nose, K. Hatta, A. Kawakami, and M. Takeichi. 1987. Calcium-dependent cell–cell adhesion molecules (cadherins): subclass specificities and possible involvement of actin bundles. *J. Cell Biol.* 105:2501–2510.
Horwitz, A., K. Duggan, C. Buck, M. Beckerle, and K. Burridge. 1986. Interaction of plasma membrane fibronectin receptor with talin—a transmembrane linkage. *Nature (Lond.).* 320:531–533.
Horwitz, A., K. Duggan, R. Greggs, C. Dekker, and C. Buck. 1985. The cell substrate attachment (CSAT) antigen has properties of a receptor for laminin and fibronectin. *J. Cell Biol.* 101:2134–2144.
Hudson, L, and F. C. Hay. 1980. Isolation and structure of immunoglobulins. *In* Practical Immunology. Blackwell Scientific Publications, Inc., Boston. 156–202.
Hynes, R. O. 1987. Integrins: a family of cell surface receptors. *Cell.* 48:549–554.
Kromer, L. F., and C. J. Cornbrooks. 1986. Transplants of Schwann cell cultures promote axonal regeneration in the adult mammalian brain. *Proc. Natl. Acad. Sci. USA.* 82:6330–6334.
Lagenauer, C., and V. Lemmon. 1987. A L1-like molecule, the 8D9 antigen, is a potent substrate for neurite extension. *Proc. Natl. Acad. Sci. USA.* 84: 7753–7757.
Lander, A. D., D. K. Fujii, D. Gospodarowicz, and L. F. Reichardt. 1982. Characterization of a factor that promotes neurite outgrowth: evidence linking activity to a heparan sulfate proteoglycan. *J. Cell Biol.* 94:574–585.
Lander, A. D., D. K. Fujii, and L. F. Reichardt. 1985. Laminin is associated with the neurite outgrowth-promoting factors found in conditioned media. *Proc. Natl. Acad. Sci. USA.* 82:2183–2187.
Lemmon, V., and S. McCloon. 1986. The appearance of an L1-like molecule in the chick primary visual pathway. *J. Neurosci.* 6:2987–2994.
Lemmon, V., E. B. Staros, H. E. Perry, and D. I. Gottlieb. 1982. A monoclonal antibody which binds to the surface of chick brain cells and myotubes: cell selectivity and the properties of the antigen. *Dev. Brain Res.* 3:349–360.
Lindholm, D., R. Heumann, M. Meyer, and H. Thoenen. 1987. Interleukin-1 regulates Nerve Growth Factor (NGF) synthesis in the non-neuronal cells of the rat sciatic nerve. *Nature (Lond.).* 330:658–659.
Manthorpe, M., E. Engvall, E. Ruoslahti, F. M. Longo, G. E. Davis, and S. Varon. 1983. Laminin promotes neuritic regeneration from cultured peripheral and central neurons. *J. Cell Biol.* 97:1882–1890.
Martini, R., and M. Schachner. 1986. Immunoelectron microscopic localization of neural cell adhesion molecules (L1, N-CAM, and MAG) and their shared carbohydrate epitope and myelin basic protein in developing sciatic nerve. *J. Cell Biol.* 103:2439–2448.
McMahan, U. J., D. R. Edgington, and D. P. Kuffler. 1980. Factors that influence regeneration of the neuromuscular junction. *J. Exp. Biol.* 89:31–42.
Neff, N. T., C. Lowrey, C. Decker, A. Tovar, C. Damsky, C. Buck, and A. F. Horwitz. 1982. A monoclonal antibody detaches embryonic skeletal muscle from extracellular matrices. *J. Cell Biol.* 95:654–666.
Neugebauer, K. M., J. M. Tomaselli, J. Lilien, and L. F. Reichardt. 1988. N-cadherin, N-CAM, and integrins promote retinal neurite outgrowth on astrocytes in vitro. *J. Cell Biol.* In press.
Nieke, J., and M. Schachner. 1985. Expression of the neural cell adhesion molecules L1 and N-CAM and their common carbohydrate epitope L2/HNK-1 during development and after transection of the mouse sciatic nerve. *Differentiation.* 30:141–151.
Noakes, P. G., and M. R. Bennett. 1987. Growth of axons into developing muscles of the chick forelimb is preceded by cells that stain with Schwann cell antibodies. *J. Comp. Neurol.* 259:330–347.
Noble, M., M. Albrechtsen, C. Moller, J. Lyles, E. Bock, C. Goridis, M. Watanabe, and U. Rutishauser. 1985. Glial cells express N-CAM/D2-CAM-like polypeptides in vitro. *Nature (Lond.).* 316:725–728.
Omlin, F. X., J. M. Mattieu, E. Philippe, J. M. Roch, and B. Droz. 1985. Expression of myelin-associated glycoprotein by small neurons of the dorsal root ganglion in chickens. *Science (Wash. DC).* 227:1359–1360.
Pollerberg, E., M. Schachner, and J. Davoust. 1986. Surface mobilities of two molecular weight forms of the neural cell adhesion molecule N-CAM are characteristic of distinct differentiation stages. *Nature (Lond.).* 324:462–465.
Poltorak, M., R. Sadoul, G. Keihauer, C. Landa, T. Fahrig, and M. Schachner. 1987. Myelin-associated glycoprotein, a member of the L2/HNK-1 family of neural cell adhesion molecules, is involved in neuron–oligodendrocyte and oligodendrocyte–oligodendrocyte interaction. *J. Cell Biol.* 105:1893–1899.
Porter, S., M. B. Clark, L. Glaser, and R. P. Bunge. 1986. Schwann cells stimulated to proliferate in the absence of neurons retain full functional capacity. *J. Neurosci.* 6:3070–3078.
Quarles, R. H. 1984. Myelin-associated glycoprotein in development and disease. *Dev. Neurosci.* 6:285–303.
Raper, J. A., M. J. Bastiani, and C. S. Goodman. 1984. Pathfinding by neuronal growth cones in grasshopper embryos. IV. The effects of ablating the A and P axons upon the behavior of the G growth cones. *J. Neurosci.* 4:2329–2345.
Rathjen, F., and M. Schachner. 1984. Immunocytological and biochemical characterization of a new neuronal cell surface component (L1 antigen) which is involved in cell adhesion. *EMBO (Eur. Mol. Biol. Organ.) J.* 3:1–10.
Rathjen, F. G., J. M. Wolff, R. Frank, F. Bonhoeffer, and U. Rutishauser. 1987. Membrane glycoproteins involved in neurite fasciculation. *J. Cell Biol.* 103:343–353.
Rogers, S. L., P. C. Letourneau, S. L. Palm, J. McCarthy, and L. T. Furcht. 1983. Neurite extension by peripheral and central nervous system neurons in response to substratum-bound fibronectin and laminin. *Dev. Biol.* 98: 212–220.

Role, L. W., and G. D. Fischbach. 1987. Changes in the numnber of chick ciliary ganglion neuron processes with time in cell culture. *J. Cell Biol.* 103: 363–370.

Ruoslahti, E., and M. D. Pierschbacher. 1987. New perspectives in cell adhesion: RGD and integrins. *Science (Wash. DC).* 238:491–497.

Salzer, J. L., W. P. Holmes, and D. R. Colman. 1987. The amino acid sequences of the myelin-associated glycoproteins: homology to the immunoglobulin gene superfamily. *J. Cell Biol.* 104:957–965.

Seilheimer, B., and M. Schachner. 1988. Studies of adhesion molecules mediating interactions between cells of peripheral nervous system indicate a major role for L1 in mediating sensory neuron growth on Schwann cells in culture. *J. Cell Biol.* 107:341–351.

So. K.-F., and A. Aguayo. 1985. Lengthy regrowth of cut axons from ganglion cells after peripheral nerve transplantation into the retina of adult rats. *Brain Res.* 328:349–354.

Sternberger, N. H., R. Quarles, Y. Itoyama, and H. F. Webster. 1979. Myelin-associated glycoprotein demonstrated immunocytochemically in myelin and myelin-forming cells of developing rat. *Proc. Natl. Acad. Sci. USA.* 76: 1510–1514.

Tomaselli, K. J., C. H. Damsky, and L. F. Reichardt. 1987. Interactions of a neuronal cell line (PC12) with laminin, collagen IV, and fibronectin: identification of integrin-related glycoproteins involved in attachment and process outgrowth. *J. Cell Biol.* 105:2347–2358.

Tomaselli, K. J., K. N. Neugebauer, J. L. Bixby, J. Lilien, and L. F. Reichardt. 1988. N-cadherin and integrins: two receptor systems that mediate neurite outgrowth on astrocyte surfaces. *Neuron.* 1:33–43.

Tomaselli, K. J., L. F. Reichardt, and J. L. Bixby. 1986. Distinct molecular interactions mediate neuronal process outgrowth on extracellular matrices and nonneuronal cell surfaces. *J. Cell Biol.* 103:2659–2672.

Volk, T., O. Cohen, and B. Geiger. 1987. Formation of heterotypic adherens-type junctions between L-CAM-containing liver cells and A-CAM-containing lens cells. *Cell.* 50:987–994.

Watanabe, M., J. L. Frelinger III, and U. Rutishauser. 1986. Topography of N-CAM structural and functional determinants. I. Classification of monoclonal antibody epitopes. *J. Cell Biol.* 103:1721–1728.